

Claims

1. A transposon TnKGloxP, characterized in comprising outer
end transposase recognition sequences having a base sequence of
5 SEQ ID NO: 3 on one end, its reverse-complementary sequence on the
other end, loxP site expressed as SEQ ID NO: 4, Km^R gene expressed
as SEQ ID NO:5 and GFP gene expressed as SEQ ID NO:6.

2. The transposon TnKGloxP according to Claim 1, chracterized
10 in comprising the base sequence of SEQ ID NO:1.

3. A transposon TnCloxP characterized in comprising outer end
transposase recognition sequences having a base sequence of SEQ ID
NO: 3 on one end, its reverse-complementary sequence on the other
15 end, loxP site expressed as SEQ ID NO:4 and Cm^R gene expressed as
SEQ ID NO:7.

4. The transposon TnCloxP according to Claim 3, characterized
in comprising the base sequence SEQ ID NO:2.

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5. A method for constructing novel strains containing deletion of
a specific chromosomal site, characterized in comprising the steps of:

(1) preparing two transposons comprising outer end
transposase recognition sequences, loxP site and different
25 selectable markers;

- (2) inserting the above two transposons, respectively, into random positions of different microbial chromosomes and determining the each inserted sites;
- (3) integrating the two microbial chromosomes by P1 phage transduction to position the two transposons comprising different selectable markers on one chromosome; and
- (4) deleting a chromosomal site between the two loxP sites by expressing Cre gene through Cre expression vector introduced.

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6. The method for constructing novel strains according to Claim 5, wherein the above two transposons are transposon TnKGloxP according to Claim 1 or 2, or transposon TnCloxP according to Claim 3 or 4.

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7. The method for constructing novel strains according to Claim 5, wherein,

- transponon TnKGloxP comprising a base sequence of SEQ ID NO:1 is prepared by preparing a vector pKGloxP by inserting GFP gene into a linear pKKloxP vector having Km^R and loxP using ligase; separating a DNA fragment comprising Km^R, GFP and loxP sites by treating the above pKGloxP vector with restriction enzyme; preparing pTnKGloxP vector by inserting the above separated DNA fragment into the linear pMODTM<MCS> vector using ligase; performing PCR of the above pTnKGloxp vector, and

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- transposon TnCloxP comprising base a sequence of SEQ ID NO: 2 is prepared by separating a DNA fragment comprising Cm^R and loxP sites by treating the above pKGloxP vector comprising Cm^R and loxP sites with restriction enzyme; preparing pTnCloxP vector by
5 inserting the above separated DNA fragment into the linear pMODTM<MCS> vector using ligase; and performing PCR of the above pTnCloxP vector.

8. The method for constructing novel strains according to Claim
10 5 or 7, characterized in additionally comprising the steps of:

- selecting two mutants from the mutants containing deletion of a specific chromosomal site, and performing P1 phage transduction using one of the selected mutants as the donor and the other as recipient, to constructing a new mutant containing
15 all chromosomal deletion sites of the above two mutant;
- using the above obtained mutant again as P1 phage recipient, and the already prepare mutant containing deletion of a specific chromosomal site as donor to perform P1 phage transduction continuously and repeatedly; and
- 20 - removing the chromosomal deletion site of other donor mutant from the chromosome of the obtained mutant continuously to reduce the chromosome of the obtained mutant by degrees.